Article

In extruded feline diets, thiamine degraded at a similar rate when stored at −20°C, compared to room temperature

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Abstract — Thiamine is an essential dietary nutrient in cats; however, studies on the stability of thiamine in pet food are limited. The objective of this study was to analyze thiamine concentrations in commercial feline extruded diets over time at room and freezing temperatures. Twelve diets were split in half and thiamine concentrations were assessed using fluorometry. One half of each diet was then stored at room temperature (24°C) and the other half was frozen (−20°C). Subsamples were analyzed at 2 other time points at 6-month intervals up to 1 year. Data were assessed using a mixed procedure (2-factor factorial model with factors time and treatment). Based on F-tests, thiamine concentrations decreased over time (P = 0.001), with no treatment*time interaction (P = 0.9534). In conclusion, regardless of treatment, thiamine degraded at a similar rate over time.

Résumé — Dans les aliments extrudés pour félins, la thiamine s'est dégradée à un taux similaire lorsqu'elle est conservée à −20 °C par rapport à la température ambiante. La thiamine est un nutriment alimentaire essentiel chez les chats; cependant, les études sur la stabilité de la thiamine dans les aliments pour animaux de compagnie sont limitées. L'objectif de la présente étude était d'analyser les concentrations de thiamine dans les aliments extrudés commerciaux pour félins au fil du temps à des températures ambiantes et de congélation. Douze aliments ont été divisés en deux et les concentrations de thiamine ont été évaluées par fluorométrie. La moitié de chaque aliment a ensuite été conservée à température ambiante (24 °C) et l'autre moitié a été congelée (−20 °C). Les sous-échantillons ont été analysés à deux moments supplémentaires à des intervalles de 6 mois jusqu'à 1 an. Les données ont été évaluées à l'aide d'une procédure mixte (modèle factoriel à deux facteurs avec les facteurs temps et traitement). Les tests F globaux ont montré une diminution de la concentration de thiamine au fil du temps (P = 0,001) et aucune interaction entre le traitement et le temps (P = 0,9534). En conclusion, quel que soit le traitement, la thiamine s'est dégradée à un rythme similaire dans le temps.

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Introduction

Thiamine (vitamin B1) is a water-soluble vitamin that is naturally present in plant- and animal-based food sources including meat (beef, pork, fish), whole grains (brown rice, bran), and legumes. Thiamine is highly susceptible to degradation during processing through exposure to heat, neutral or alkaline pH, and oxidation (1–4). The presence of anti-thiamine compounds such as thiamine-degrading enzymes (in raw fish) and sulfites (used in meat preservation) can also result in large losses of the vitamin (5). To compensate for these losses, processed human foods such as breads and cereals are commonly fortified with a synthetic form of the vitamin (thiamine mononitrate) (1).

Thiamine is crucial in various biochemical pathways including glucose and amino acid metabolism, and neurotransmission (central and peripheral nervous system). Signs of deficiency can be vague and acute, related to heart and nervous system function, and can have life-threatening consequences (6). Thiamine is an essential nutrient in dogs and cats, as they cannot synthesize the vitamin. Therefore, they must consume adequate amounts of thiamine from their diet to prevent development of thiamine deficiency (6,7).

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For formulation of diets, Canadian guidelines for industry follow the Association of American Feed Control Officials (AAFCO) recommendations, which are historically based on recommendations provided by the United States National Research Council (US NRC). According to thiamine requirements set forth by the US NRC, adult cats have a minimum adequate intake and minimum recommended allowance of 4.4 and 5.6 mg/kg, respectively on a dry matter basis (DMB) (8). There is currently no known safe upper limit for thiamine intake, due to lack of data (8). The AAFCO recommendation for commercial adult cat food is a minimum of 5.6 mg thiamine/kg daily on a DMB (9).

Thiamine deficiency in canned feline diets has recently become an area of concern; in 1 study, 13.3% of canned feline diets tested had thiamine concentrations below the AAFCO recommendation at the time of the study, despite having a nutritional adequacy statement indicating the diets were complete and balanced (10). In North America there have been numerous recalls of commercial pet foods due to thiamine deficiency involving canned, extruded, and raw feline diets (11). This may be due to thiamine's susceptibility to degradation during processing and storage conditions (1–4).

Pet owners may opt to purchase pet food in bulk as it is typically cheaper; however, cats may not consume the large quantity of food before it loses freshness or before the expiration date. Therefore, to preserve freshness and palatability, owners may choose to freeze the food for use later rather than store at the recommended room temperature. To the authors' knowledge, the potential for thiamine degradation in extruded feline diets stored at ambient and freezing temperatures over time has not been investigated. The objective of this study, therefore, was to examine rates of thiamine degradation in commercial extruded feline diets after 6- and 12-months of storage at freezing and room temperatures. It is hypothesized that because thiamine is sensitive to processing and the environment, freezing and thawing kibble may increase thiamine losses compared to room temperature storage, and that thiamine will degrade steadily with time.

Materials and methods

Diets

Twelve commercially available extruded feline diets were assessed in this study. Selection of diets was based on poultry as the major protein source, to avoid thiamine-degrading enzymes present in other ingredients, such as fish (1). Diets were purchased from pet stores in Guelph and Hamilton, Ontario, Canada in June 2017 with expiration dates no earlier than June 2018. Labeling information such as the AAFCO nutritional adequacy statement, guaranteed analyses, and ingredient lists were recorded.

Sampling and storage

Samples were obtained from 12 extruded feline diets following their purchase. Each diet was split in half after purchase. Subsequently, 1/2 of each diet was stored at room temperature (24°C) and the other half was frozen at −20°C. All samples were stored in airtight bags and room temperature samples were placed in opaque storage containers out of direct sunlight. The baseline subsamples were taken immediately after separation of the diets, before storage and treatment. Following this, subsamples of each half were analyzed at 2 additional time points in 6-month intervals (6- and 12-months). Frozen samples were thawed at room temperature before processing. All samples were taken in duplicate, equalling 48 samples per time point.

Analytical methods

All samples were sent to a commercial laboratory (Maxxam Analytics, Mississauga, Ontario), where thiamine and moisture concentrations were assessed according to AOAC methodologies 942.23 and 984.25, respectively (12).

Statistical analysis

Analyses were conducted using commercial statistical software (SAS 9.4; SAS Institute, Cary, North Carolina, USA). Assumptions of the analysis of variance (ANOVA) were assessed with examination of the residuals and a Shapiro-Wilk test. Non-normal data were log-transformed and assessed using a mixed procedure. The design was a 2-factor factorial method with time and treatment as factors. The main effects and 2-way interactions were included in the initial model with treatment, time, and treatment*time. Diets were included as random factors in the model to account for differing thiamine concentrations. Replication came from the 2 measurements at each treatment and time combination. Sampling was done destructively at each time point; therefore, no repeated measures occurred. Data are expressed as geometric medians (back-transformed logs) with upper and lower limits.

Results

Seven diets were formulated to meet the AAFCO nutrient profiles for maintenance of adult cats, and 5 diets were formulated to meet the AAFCO nutrient profiles for all life stages of cats. Guaranteed analyses for all diets are presented in Table 1, and the average baseline sample analysis of moisture (%) and thiamine content (mg/kg) are presented in Table 2. Although all diets were manufactured following the AAFCO's 2017 increase in dietary thiamine recommendations, 1 diet did not meet the minimum recommendation at any time point throughout the study and was subsequently removed from statistical analysis.

Based on overall F-tests, there were effects of treatment (P = 0.017) and time (P = 0.0010). However, there was no treatment*time interaction (P = 0.9534). Thiamine significantly decreased over time, regardless of treatment (Figure 1); baseline to 12 mo (P = 0.0052) and 6 to 12 mo (P = 0.0004). No differences were noted between baseline and 6 mo (P = 0.4164). Freezing temperature samples (26.28 mg/kg DMB) had significantly more thiamine than room temperature samples (24.68 mg/kg DMB) regardless of time (Figure 2).

Discussion

Nutritional adequacy statements for all 12 diets indicated they were formulated to meet the AAFCO recommendations for feline maintenance or all life stages (9). It was anticipated that the diets would contain various thiamine concentrations;

Table 1. Guaranteed analyses and AAFCO nutritional adequacy statement of 12 feline extruded diets on an as fed basis reported by manufacturer.

Diet	AAFCO	CP, % min	CF, % min	CFr, % total	Moisture, % max	ME (kcal/kg)
a	Adult cat	32.0	15.5	3.0	10.0	3710
b	Adult cat	40.0	13.5	4.8	12.0	4037
c	Adult cat	33.8	19.3	9.1	8.0	3702
d	Adult cat	38.0	16.0	6.0	10.0	3713
e	All life stages	30.0	11.0	4.0	12.0	3500
f	Adult cat	40.0	14.0	7.5	11.0	3580
g	Adult cat	40.0	18.0	4.0	10.0	3907
h	All life stages	30.0	20.0	3.0	10.0	4008
i	All life stages	43.0	19.5	3.0	9.0	4150
j	Adult cat	30.0	11.0	4.5	10.0	3300
k	All life stages	37.0	20.0	3.0	10.0	4020
l	All life stages	32.0	20.0	2.5	10.0	4604

AAFCO — Nutritional Adequacy Statement, Association of American Feed Control Officials; CP — crude protein; CF — crude fat; CFr — crude fiber; ME — metabolizable energy; min — minimum; max — maximum.

Table 2. Analysis of moisture (%) and thiamine content (mg/kg) of 12 feline extruded diets attained through averaging 4 baseline samples analyzed by a commercial laboratory.

	Laboratory analyses		Calculated
Diet	Moisture (%)	Thiamine as fed (mg/kg)	Thiamine DMB (mg/kg)
a	6.1	16.8	17.8
b	5.7	192.5	204.2
c	5.9	34.3	36.4
d	5.5	26.5	28.0
e	7.7	16.0	17.3
f	4.5	19.5	20.4
g	5.5	25.8	27.2
h	3.9	32.5	33.8
i	5.2	29.8	31.4
j	6.9	6.3	6.7
k	7.5	2.5	2.7
l	4.6	13.8	14.4

DMB — Dry matter basis.

however, 1 diet contained less than half the minimum recommended amount of thiamine according to the AAFCO 2017. The deficient diet was void of any known thiamine-degrading ingredients and was purchased at least 12 mo before the expiration date. Only 1 bag of the diet was tested; therefore, it is unknown if this was an isolated incident or if other bags were affected. Regardless, thiamine deficiency in a single bag is cause for concern, and the manufacturer was contacted with this important information. Conversely, another diet contained almost 30-fold the minimum amount of thiamine recommended by the AAFCO (9). The high concentration may be the manufacturer's response to previous pet food recalls related to thiamine deficiency and likely results in an increased manufacturing cost.

For most cats, the sole source of nutrition is commercial pet food. Thiamine is an essential nutrient in cats; thus, cats fed a diet deficient in thiamine are at risk of developing a thiamine deficiency. Clinical signs of deficiency can occur within 2 wk of being fed a deficient diet, and cats present with anorexia, vomiting, seizures/convulsions, weakness, prostration, and if left untreated, eventually death (8,13–15). It is unknown if high dietary thiamine concentrations pose any benefits or risks to cats (8). Although thiamine is a water-soluble vitamin, whether a safe upper limit for dietary thiamine in cats exists is unknown and more research is required.

This study reported a significant effect of storage time on thiamine concentration in extruded cat food. Given that thiamine is a labile vitamin, it is easily degraded in foods when exposed to various elements (pH, oxidation, UV light) and storage conditions (ambient temperature, heat, freezing) (1,3,4). Significant thiamine loss occurs over time (10 d to 12 mo) when foods, such as spinach, canned tomatoes, and peaches, are stored at room temperature (16–18). Thiamine in food is particularly susceptible to degradation during processing due to exposure to heat, having reported losses of 50 to 85% with pelleting or extrusion and 50 to 60% with household cooking (19–21). The findings of the present study indicated that over a 12-month storage period, thiamine concentrations decreased over time at both room and freezing temperatures. On average, there was approximately 95% retention of thiamine from baseline to 12 mo for both treatments. Thus, the clinical relevance of the decline may be inconsequential as thiamine loss was low and no diet previously above the AAFCO recommendations became deficient. However, increased loss of thiamine may be expected with more prolonged storage.

That frozen samples had significantly higher thiamine concentrations throughout the study compared to room temperature samples is difficult to interpret since baseline samples were taken from the same bag and collected before treatment was applied, yet, baseline concentrations were significantly different. Based on this finding, combined with no significant treatment*time interaction (P = 0.9534), we inferred that, regardless of treatment, thiamine concentrations degraded at a similar rate over time and there is no difference between treatments.

Frozen storage of raw and processed foods is one of the fundamental methods of preservation for palatability and freshness. Thiamine stability in regard to storage at freezing temperatures has received little attention in the literature, and results are difficult to interpret, as the effects of both freezing and at least 1 other cooking method (blanching, canning, cooking) are

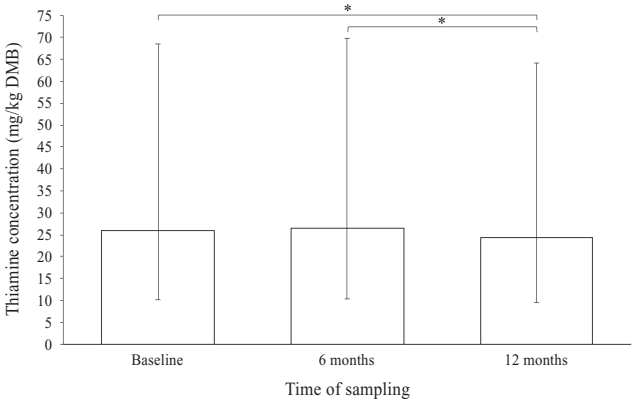

Figure 1. Thiamine concentrations (mg/kg DMB) of extruded diet samples analyzed at baseline, 6 mo, and 12 mo. Each bar represents the geometric median (back-transformed log) thiamine concentration of all diet samples for both treatments (room temperature and −20°C) per time point ($n = 11$) ± upper and lower limits from all diets that met the AAFCO recommendation. Significance at $P < 0.05$ is marked with an asterisk (*). There were overall effects of storage time ($P = 0.0010$), baseline to 12-mo ($P = 0.0052$) and 6 to 12 mo ($P = 0.0004$). However, no differences were noted between baseline and 6 mo ($P = 0.4164$).

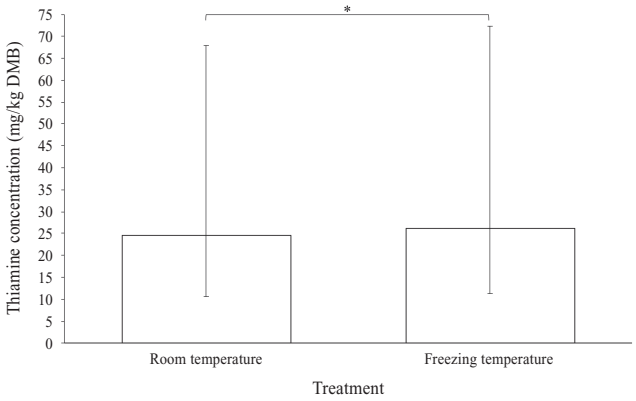

Figure 2. Thiamine concentrations (mg/kg DMB) of extruded diet samples stored at room temperature and at −20°C. Each bar represents the geometric median (back-transformed log) thiamine concentration of all diet samples for all time points (baseline, 6 mo and 12 mo) per treatment ± upper and lower limits from all diets that met the AAFCO recommendation ($n = 11$). Significance at $P < 0.05$ is marked with an asterisk (*). There was an overall effect of treatment ($P = 0.0170$).

examined in many studies (22–25). In long-term freezer storage of meats, there were varying thiamine losses over time in chicken breast and legs, pork chops, pork loins, lamb chops, and beef patties (26–30). Based on assessments of frozen food over time, there are some indications that thiamine concentration may initially increase during storage or processing before declining, yet no mechanisms to explain this have been proposed (18,30–32).

Although there is much research on thiamine degradation in human foods during processing and various storage conditions, there is limited research on pet foods, especially on extruded diets. Mooney et al (33) reported more than 90% thiamine loss when exposed to an elevated temperature (50°C) for a 24-week interval, in order to mimic heat stress due to transport, although thiamine concentrations appeared to be relatively stable over a 24-month period in extruded pet food stored in environmentally controlled conditions at ambient temperature (20 to 22°C). In contrast, a summary of previous research examining thiamine retention in extruded dog and cat foods reported that retention after 18 mo of storage was 57.5% (34). Though a decrease in thiamine concentration over time was noted, storage conditions were not described. To the authors' knowledge, there is currently no research investigating the effect of freezing on thiamine concentration in extruded cat foods, which was the focus of this study.

To determine the mechanism of action responsible for changes in thiamine concentrations in commercial extruded pet foods over time and under various storage conditions, a broader analysis of pet food is required, and limitations of this study's design must be addressed. Future studies should sample at more time points for a longer study interval, collect samples from multiple bags and batches per diet, and analyze thiamine concentration in triplicate to account for variation with duplicate samples. Ideally 2 laboratory methods for thiamine analysis should be employed; however, this study used the most widely accepted method of thiamine analysis in commercial food, measuring thiamine concentration by fluorescence with a fluorimeter (AOAC 942.23; AOAC, Rockland, Maryland, USA). This method has specific quantification with a reportable detection limit of 0.01 mg/100 g (35). This study also stored room temperature samples in airtight opaque storage bins out of direct sunlight to limit the degradation of thiamine. Pet owners, however, may not store their pet's food under such conditions.

Current recommendations for storage of extruded pet food are to keep the food in its original package (to ensure lot and batch information is available in the event of a recall), within an airtight container (to prevent pest infestation), at room temperature, and out of direct sunlight (to ensure a stable temperature). In the present study there was a similar degradation of thiamine over time in both room temperature and freezing storage; however, further research is required to make recommendations for or against freezing extruded pet foods with respect to nutrient degradation or preservation. CVJ

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